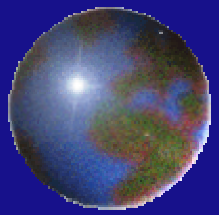
Vaccine Cell Substrates 2004



Adventitious Virus Tests

Rebecca Sheets, Ph.D.,
Commander, USPHS
NIH/NIAID

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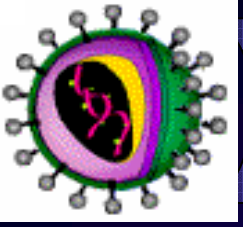
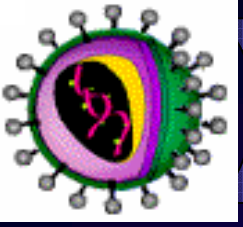


Cell Substrate Characterization

- Each manufacturer characterizes the cell substrates banked and used in production in their facility
  - history of isolation and banking
  - growth characteristics
  - karyology, identity, and tumorigenicity
  - freedom from adventitious agents

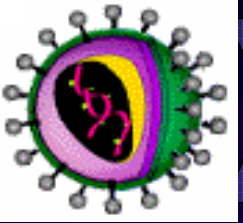
Tests to Characterize Cell Banks

- Karyology
- Isoenzyme analysis
- Tumorigenicity
  - tumor formation (progressing nodules and/or metastases) in immunosuppressed rodents
  - colony formation in soft agar
  - not necessary for cells of rodent origin (as all continuous cell lines of such are tumorigenic by in vivo assay)
  - other cells expected to pass tumorigenicity testing



Adventitious Agent Tests

- Bacterial and fungal sterility (610.12)
- Mycoplasma (& spiroplasma)
- Mycobacteria (animals/culture-650.13)
- Viruses (*in vitro*, *in vivo*)
  - Acute (Lytic, Hemadsorbing/Hemagglutinating)
  - Latent (e.g., retroviruses or other oncogenic viruses)
- Retroviruses
- Specific tests (PCR)
- Animal-derived raw materials
  - 9 CFR 113 tests
  - from BSE-free countries

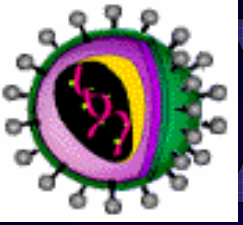


Adventitious Virus Tests

- ***In Vitro* tests**
  - monolayers of at least 3 cell types
    - same species, tissue as substrate
    - human diploid cells
    - monkey kidney cells
  - tests for hemadsorption and hemagglutination at end of culture period
- **Animal-derived raw materials**
  - 9 CFR 113 tests
  - from BSE-free countries

Adventitious virus tests (2)

- *In Vivo* tests
  - adult and suckling mice
  - embryonated hens' eggs
  - when appropriate
    - guinea pigs
    - rabbits
    - monkeys



Adventitious Virus Tests (3)

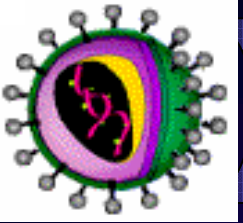
- **For rodent substrates**
  - MAP, RAP, HAP - antibody production tests
  - Lymphocytic choriomeningitis virus
- **For human substrates**
  - EBV, CMV
  - HBV, HCV
  - *in vitro* techniques
  - tissue source and donor medical history

Adventitious Virus Tests (4)

- If appropriate
  - papillomaviruses
  - adenoviruses
  - Herpes Virus Type 6 (HHV-6), others
- Retroviruses
  - Transmission Electron Microscopy
  - Reverse Transcriptase assays
  - Infectivity assays

Where did it start?

- One of the earliest class of cell-culture derived vaccines was poliovirus vaccines
- March 16, 17, 1961
  - Hearing before a subcommittee on interstate and foreign commerce (committee of the House of Representatives)
  - Initial proposed regulations for the manufacture of OPV
  - These regs ultimately became part of 21 CRF 630's

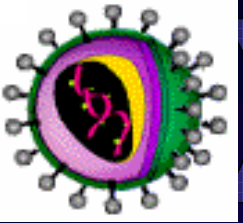


Tests for polioviruses harvests

- In 1961, poliovirus harvests were intended to be tested for “adventitious and other infectious agents including polioviruses of other types or strains, simian agents, Mycobacterium tuberculosis, pox viruses, lymphocytic choriomeningitis virus, Echoviruses, coxsackieviruses, and B-virus.”

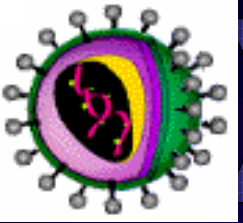
Specific Tests Required

- Rabbits
- Adult mice
- Suckling mice
- Guinea Pigs
- Monkey Kidney Tissue Cultures
- Human Tissue Cultures
- Rabbit Kidney Tissue Cultures



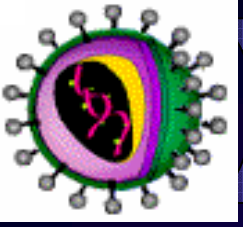
What were these tests for?

- Rabbits – B-virus
- Rabbit Kidney Tissue Cultures – B-virus
- These tests are not routinely (although sometimes) included for most vaccines, so methods won't be elaborated



Adult Mice

- LCMV or other viruses
- ≥20 adult mice
- i.p. with 0.5 mL, i.c., with 0.03 mL
- Mice must survive 21 days
  - ≥80% survival
  - No sign of viral infection
- Capable to detect LCMV, coxsackieviruses, flaviviruses, rabies

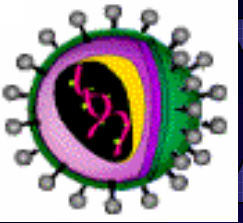


Suckling Mice

- Coxsackieviruses (particularly type A)
- ≥20 mice less than 24 hours old
- i.c., 0.01 mL, i.p., 0.1 mL
- 14 days
- Subinoculation into additional mice for 14 days
- Mice must survive
  - ≥80% survival both inoculations
  - No signs of viral infection
- Capable of detecting coxsackieviruses, other picornaviruses (polioviruses, echoviruses), alphaviruses, herpesviruses (HSV), flaviviruses, rabies, many murine agents, others

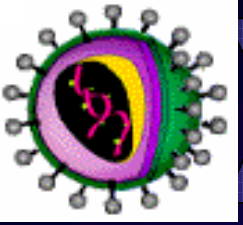
Guinea Pigs

- LCMV, M. Tb
- ≥5 guinea pigs
- 0.1 mL i.c., 5 mL i.p.
- 42 days
- ≥80% survival
  - No signs of LCMV or M. Tb infection
- Capable of detecting paramyxoviruses, reoviruses, filoviruses



Tissue Cultures

- Monkey Kidney Cells (simian agents)
- Human Diploid Cells (originally to detect measles)
- Same species, tissue as production
- 14 days, subculture for additional 14 days
- CPE, hemadsorption/hemagglutination (RBCs of guinea pigs, chickens, and human or rhesus monkey – 2 temperatures, ½ each)
- Capable of detecting a wide array of viruses



Egg tests

- 0.5 mL allantoic route
  - Orthomyxoviruses (influenzaviruses), paramyxoviruses (mumps, measles, parainfluenzaviruses), alphaviruses,
- 0.5 mL yolk sac route
  - Herpesviruses (HSV), poxviruses, rhabdoviruses, rickettsiae, mycoplasmas, bacteria